UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/991,448	11/16/2001	Robert McDaniel	300622005210	3975

25225 7590 10/22/2003

MORRISON & FOERSTER LLP
3811 VALLEY CENTRE DRIVE
SUITE 500
SAN DIEGO, CA 92130-2332

EXAMINER	
KERR, KATHLEEN M	
ART UNIT	PAPER NUMBER
1652	

DATE MAILED: 10/22/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/991,448	MCDANIEL, ROBERT	
	Examiner	Art Unit	
	Kathleen M Kerr	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 October 2002* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-17* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-17* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *8* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☒ Other: *LIGAND* .

DETAILED ACTION

Application Status

1. Claims 1-17 are pending by virtue of a preliminary amendment filed that cancelled Claims 18-20. Claims 1-17 will be examined herein.

Priority

2. The instant application is granted the benefit of priority for U.S. non-provisional application Nos. 09/768,927 (divisional parent) and 09/428,517 (CIP parent) filed on January 23, 2001 and October 28, 1999, respectively. The application is also granted benefit of priority for U.S. provisional application Nos. 60/177,660 and 60/120,254 filed on January 27, 2000 and February 16, 1999, respectively. The application is also granted benefit of priority for PCT/US99/24478.

The instant application is not granted the benefit of 60/106,100 since said application and the instant application lack a common inventor.

The Examiner notes that the first disclosure of methods using a generic P_{450} monooxygenase (Claims 1-4 and 6-11) is found in 09/768,927 filed on January 23, 2001. Claim 5 has priority back to February 16, 1999; and Claims 12-17 have priority back to January 27, 2000 (see explanation below).

Provisional application 60/120,254 filed February 16, 1999 describes oleP as an epoxidase gene useful in producing compounds with an epoxide moiety (see page 40, first paragraph); while epoxide moieties can be considered precursors to hydroxyl moieties, no

description of generic P_{450} monooxygenases, other PKS P_{450} monooxygenases, or oleP as a P_{450} monooxygenase is found in this application.

Similarly for 09/428,517 filed October 28, 1999 (USPN 6,251,636 which is also PCT/US99/24478), oleP is described for use in host cells to produce a polyketide with a C-8-C-8a epoxide and more general hydroxylation at C6 and/or C12 is described using eryF, picK, or eryK genes (see columns 83-84). Nothing in 09/428,517 provides support for use of generic P450 monooxygenases (Claims 1-4 and 6-11), in fact the term is not used, and nothing supports using host cells to the exclusion of *S. antibioticus* (Claims 12-17).

By far the most extensive oleP priority document is provisional application 60/177,660 filed January 27, 2000, which describes OleP as a P_{450} monooxygenase (see page 3). This document also describes other monooxygenases in the PKS field from the erythromycin, picromycin and tylosin gene clusters (see page 6); although the invention is specific for using OleP. No generic description of using general monooxygenases or even P_{450} monooxygenases from PKS gene clusters is found in the specification, the Abstract, or the claims – all of these are specific to using OleP. Claims 12-17 are supported, however, in the claims presented in 60/177,660.

Information Disclosure Statement

3. The information disclosure statement filed on October 15, 2002 (Paper No. 8) has been reviewed, and its references have been considered as shown by the Examiner's initials next to each citation on the attached copy.

Compliance with the Sequence Rules

4. This application contains sequence disclosures (see page 13, lines 12 and 25-26) that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 C.F.R. § 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 C.F.R. § 1.821 through 1.825; applicants' attention is directed to the final rulemaking notice published at 55 FR 18230 (May 1, 1990), and 1114 OG 29 (May 15, 1990). To be in compliance, applicants must provide an initial computer readable form (CRF) copy of the "Sequence Listing", an initial paper copy of the "Sequence Listing", as well as an amendment directing its entry into the specification, and a statement that the content of the paper and CRF copies are the same and, where applicable, include no new matter as required by 37 C.F.R. § 1.821(e) or 1.821(f) or 1.821(g) or 1.821(b) or 1.825(d).

Objections to the Specification

5. The specification is objected to because the title is not descriptive. A new title is required that is clearly indicative of the invention to which the elected claims are drawn (see M.P.E.P. § 606.01). The Examiner suggests the following new title:

---Methods for Introducing Hydroxyl or Epoxide Groups into Polyketides using a P_{450} Monooxygenase---

6. In the specification, the Abstract is objected to for not completely describing the disclosed subject matter (see M.P.E.P. § 608.01(b)). It is noted that in many databases and in foreign countries, the Abstract is crucial in defining the disclosed subject matter, thus, its

completeness is essential. The Examiner suggests the inclusion of a definition of the abbreviation "OlePKS" and "DEBS" for completeness.

7. The specification is objected to for lacking complete and correct continuity data in the first paragraph.

a) A claim to the divisional parent application No. 09/768,927 filed January 23, 2001 now USPN 6,388,099 is missing.
b) The claim to the CIP parent 09/428,517 must be updated to include "now USPN 6,251,636".
c) The claim to the PCT is unclear since the full number of the application is --- PCT/US99/24478---.
d) The claim to provisional application 60/106,100 has been denied (see above) and must be removed.

Appropriate amendment to the specification is required (see M.P.E.P. § 201.11).

8. The specification is objected to for lacking updated information and/or being confusing or incomplete as follows:

a) On page 1, line 29, 09/428,517 must be updated to 6,251,636. This citation is also on page 12, line 16.
b) On page 2, line 25, "PCT 00/026,349" is confusing without a country before the "00"; and there are too many digits after the backslash. This citation is also on page 4, line 28 and page 12, lines 15-16.
c) On page 3, line 25, "monoxygenase" is misspelled; the correct spelling is --- monooxygenase---.
d) On page 5, lines 29-31, the Witkowski citation is incomplete
e) On page 6, line 6, 09/181,833 must be updated to 6,177,262. This citation is also on page 11, line 20.
f) On page 6, line 12, "PCT 99/61599" is confusing without a country before the "99".

Appropriate correction is required.

Objections to the Claims

9. Claim 1 is objected to for a misspelling; the correct spelling of "monoxygenase" is ---monooxygenase---. Correction is required.

Claim Rejections - 35 U.S.C. § 112

The following is a quotation of the second paragraph of 35 U.S.C. § 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

10. Claims 1-4 and 6-11 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The metes and bounds of the term "P450 monooxygenase" are unclear. Monooxygenase is a term of art as well as P_{450}-like monooxygenases (see attachment from LIGAND). In the specification, OleP is the only species used, and it is noted as being homologous to macrolide oxidases in the erythromycin, picromycin and tylosin gene clusters (see page 7). Is the claim limited to macrolide P_{450}-like monooxygenases or can any P450 monooxygenase be used? Clarification is required.

11. Claims 1-11 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Claim 1 is unclear as it appears to be lacking a step – that is, the requirement that the host cell produce a polyketide and/or the requirement that the host cell express a polyketide synthase. The mere expression of oleP in a host cell, the only named method step, will not produce hydroxylated polyketides. Clarification is required.

12. Claims 6-7 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The terms "**a** 6-deoxyerythronolide B synthase" and "**a** 8,8a-deoxyoleandolide synthase" (emphasis added in each) are unclear. Is there more than on 6-dEB synthase? Can variants be used? If so, to what extent must native DEBS be used? The same questions are asked for the oleandomycin PKS. Clarification is required.

13. Claims 12-17 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The abbreviation "DEBS" in Claims 12-13 must be defined upon its first appearance in the claims. Correction is required.

14. Claims 11, 13, 15, and 17 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The claims are confusing because they depend from Claim 6 (via Claim 8) which require using the erythromycin PKS (not the oleandomycin PKS as in Claim 7) yet the oleandomycin derivative is required to be produced. Moreover, in Claim 13, the DEBS PKS is required, not the oleandomycin PKS. Clarification is required.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

15. Claims 1-11, 13, 15, and 17 are rejected under 35 U.S.C. § 112, first paragraph, scope of enablement, because the specification, while being enabling for methods of introducing

hydroxyl/epoxide groups into carbon 8 or 8a or an erythromycin or oleandomycin polyketide using OleP in an appropriate host cell, does not reasonably provide enablement for methods of introducing hydroxyl/epoxide groups into *any* position of *any* polyketide using *any* P_{450} monooxygenase. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims. Claims 13, 15, and 17 are included in this rejection based on the rejection under 35 U.S.C. § 112, second paragraph above relating to the use of the erythromycin synthase to make oleandomycin derivatives. To practice the claimed methods to the full extent of their scope would require undue experimentation.

The factors to be considered in determining whether undue experimentation is required are summarized In re Wands 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988). The Court in Wands states: "Enablement is not precluded by the necessity for some experimentation such as routine screening. However, experimentation needed to practice the invention must not be undue experimentation. The key word is 'undue,' not 'experimentation.' " (Wands, 8 USPQ2d 1404). Clearly, enablement of a claimed invention cannot be predicated on the basis of quantity of experimentation required to make or use the invention. "Whether undue experimentation is needed is not a single, simple factual determination, but rather is a conclusion reached by weighing many factual considerations." (Wands, 8 USPQ2d 1404). The factors to be considered in determining whether undue experimentation is required include: (1) the quantity of experimentation necessary, (2) the amount or direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the

breadth of the claims. While all of these factors are considered, a sufficient amount for a *prima facie* case is discussed below.

In the specification, the only example specific to the claimed methods is taught in Example 5; no specifics are disclosed in this example except for the use of *S. lividans* and production of the erythronolide 8, 8a product. On page 8, an expression plasmid for both 6-dEB and 8,8a-deoxyoleandolide (to be selectively expressed) is described as being transformed into *S. lividans* wherein the *oleP* gene had been integrated into the chromosome. Piecing together page 8 with Example 5, it seems evident that either the 6dEB or oleandolide 8, 8a-hydroxyl products can be produced using the claimed method (only the 6dEB product was reduced to practice – see Table 2 for NMR data of said product), particularly since these polyketides are highly similar (see page 2 noting a methyl-ethyl substitution distinguishing the two compounds).

No working examples demonstrate incorporation of epoxides or hydroxyl groups on positions other than C8 of erythromycin-type polyketides. Stassi *et al.* (see IDS) describe how different hydroxylases are required to insert oxygen at different positions on erythromycin though they use the same mechanism (eryF for C6 and eryK for C12) (see page 186). Xue *et al.* (see IDS) describe a P_{450} hydroxylase from the picromycin PKS, PikC, that "accepts macrolide substrates of different ring sizes and catalyzes addition of a hydroxyl group at two distinct positions on a macrolactone"; however, emphasis is added that this is novel flexibility for a PKS hydroxylase(see page 666). Moreover, the Examiner notes that the "different ring sizes" are very similar and the different positions are spatially very close. Betlach *et al.* (1998 - see IDS) further emphasize the point of unpredictability of all PKS P_{450} monooxygenases working with all polyketides at all positions (see page 14942).

No working examples demonstrate epoxide and/or hydroxyl incorporation into polyketides produced by type II PKS systems, such as actinorhodin. No working examples demonstrate epoxide and/or hydroxyl incorporation into polyketides using generic P450 monooxygenases, such as cytochrome P_{450} reductase (E.C. 1.14.13.68). No direction for incorporation of epoxides or hydroxyl groups on other positions of polyketides is provided. No direction for incorporation of epoxides or hydroxyl groups onto any position of non-type I polyketides is provided. No direction for incorporation of epoxides or hydroxyl groups into polyketides using monooxygenases other than those from erythromycin, picromycin and tylosin gene clusters is provided; no direction for the identification of these monooxygenases is provided, other than the implication that they always reside in PKS gene clusters. While the state of the art has multiplied with new PKS gene clusters and their recombinant uses of late, little has been learned about the monooxygenases in the clusters, i.e., substrate specificity (or promiscuity). Thus, the ability to use any P_{450} monooxygenase to put a hydroxyl on any location of any polyketide is wholly unpredictable.

Claim Rejections - 35 U.S.C. § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent.

16. Claims 1-4 and 6-17 are rejected under 35 U.S.C. § 102(e) as being anticipated by Betlach *et al.* (see IDS, USPN 6,251,636). The instant claims are drawn to methods of introducing a hydroxyl group onto the C8 or C8a position of erythromycin and oleandomycin using a *S. lividans* host cell, that does not produce these polyketides naturally, and that expresses oleP and either DEBS or OlePKS. The Examiner notes that this USPN is a CIP parent of the instant application. As previously noted, the genus of the instant claims does not have priority back to this parent. However, the species disclosed (specific to OleP) anticipates the genus of the claims (not specific for OleP, not in *S. antibioticus*).

Betlach *et al.* teach expressing oleP in *Streptomyces* host cells in combination with expressing various PKS enzymes from PKS gene clusters, including erythromycin and oleandomycin, to produce erythromycin and oleandomycin derivatives with hydroxyl group onto the C8 or C8a position (column 76, lines 57-62, and columns 83-84, bridging paragraph). Betlach *et al.* also describe *S. lividans*, modified like CH999 *S. coelicolor*, as preferred host cells for all embodiments described throughout (see columns 65-66, bridging paragraph).

17. Claims 1-4 and 6, 8, and 10 are rejected under 35 U.S.C. § 102(a) as being anticipated by Shah *et al.* (Cloning, Characterization and Heterologous Expression of a Polyketide Synthase and P-450 Oxidase Involved in the Biosynthesis of the Antibiotic Oleandomycin. The Journal of Antibiotics (May, 2000) 53:502-508). The instant claims are drawn to methods of introducing a hydroxyl group onto the C8 or C8a position of erythromycin using a host cell, that does not produce these polyketides naturally, and that expresses oleP and DEBS.

Shah *et al.* teach expression of the DEBS gene cluster in *S. lividans* with oleP to produce 8,8a-dihydroxy-6dEB (see Abstract).

Claim Rejections - 35 U.S.C. § 103

The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

18. Claim 5 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Betlach *et al.* (USPN 6,251,636 – see IDS) in view of Rodriguez *et al.* (see IDS). The instant claims are drawn to methods of making hydroxylated polyketides by expressing OlePKS and OleP together in a host cell.

Betlach *et al.* is being used with an earliest effective filing date of October 29, 1998 – the date of 60/106,100. Betlach *et al.* teach, by way of 09/106,100 which is incorporated by reference, heterologous expression of the OlePKS in combination with a P_{450} oxidase homologue to produce polyketide derivatives (see page 14). Betlach *et al.* do not teach using OleP as said homologue.

Rodriguez *et al.* teach OleP a cytochrome P_{450}-like gene responsible for oxidation of oleandomycin (see Abstract).

It would have been obvious to combine the teachings of Betlach *et al.* and Rodriguez *et al.* because Rodriguez *et al.* describe OleP as a homologue whose use is proposed by Betlach *et al.* One would have been motivated to combine the above teachings because oleandomycin and its derivatives are useful antibiotics and general pharmaceuticals. One would have had a reasonable expectation of success that the OlePKS and OleP could be co-expressed in, for example CH999 cells proposed by Betlach *et al.* in 60/106,100, because heterologous PKS

expression has been achieved with other PKS genes in CH999 cells and with OleP in *E. coli* (see Rodriguez *et al.* Abstract).

Other References Cited

19. The following references are cited to complete the record:

a) Mendes *et al.* Engineered biosynthesis of novel polyenes. Chemistry & Biology (July, 2001) 8:635-644.
b) Gaisser *et al.* Parallel pathways for oxidation of 14-membered polyketide macrolactones in Saccharopolyspora erythraea. Molecular Microbiology (2002) 44(3):771-781.
c) Molnar *et al.* Organisation of the biosynthetic gene cluster for rapamycin in *Streptomyces hygroscopicus*: analysis of genes flanking the polyketide synthase. Gene (1996) 169:1-7.

Conclusion

20. Claims 1-17 are not allowed for the reasons identified in the numbered sections of this Office action. Applicants must respond to the objections/rejections in each of the numbered sections in this Office action to be fully responsive in prosecution.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Kathleen M Kerr whose telephone number is (703) 305-1229. The examiner can normally be reached on Monday through Friday, from 8:30am to 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathupura Achutamurthy can be reached on (703) 308-3804. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

KMK
October 17, 2003